IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Suk Cho et al. Art Unit : 1615
Serial No. : 09/800,195 Examiner : C. Evans
Filed : March 6, 2001
Title : DIETARY SUPPLEMENT COMPOSITIONS

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132 OF ERIN STONE

I, Erin Stone, declare as follows:

1. I am a citizen of the United States and presently live at 2085 Allan #4, Idaho Falls, ID, 83404.

2. I am presently employed by Melaleuca, Inc., and have been so employed since February 2003.

3. I received a B.S. degree in Biology from Idaho State University in August 2000.

4. I was responsible for performing efficacy experiments on dietary supplement formulations that included different grape seed extracts. The experiments were designed to compare the ability of the formulations to inhibit platelet aggregation. I performed the test using an *in vitro* assay for platelet aggregation according to methods previously published by Dr. Folts *et al.* ("Effect of Polyphenolic Flavonoid Compounds on Platelets," Dahnansayan Shanmuganayagam and John Folts, pages 369-380, in Methods in Enzymology, Volume 335 Flavonoids and Other Polyphenolics (2001), Lester Packer (Ed.), Academic Press, ISBN: 0-12-182236-2.) I received training on doing these experiments from Dr. Folts at the University of Wisconsin in May, 2003.

Laboratories). The solutions were prepared such that adding 4 µL of each solution to 1 mL of diluted blood would achieve the following concentrations of grape seed extract in the blood sample: for Formulation A: 380 mg/L and 760 mg/L; for Formulation B: 125 mg/L and 250 mg/L. Thus, a total of 12 samples were prepared. Each sample was sonicated (Branson 3510) for 5 minutes and then vortexed (Fisher Model # 232) for 1 minute to ensure that the sample was completely dissolved.

A 4 –Channel Whole Blood Impedance Aggregometer was used to observe the effects of the extracts on platelets. Whole blood samples were obtained from volunteers currently employed at Melaleuca, Inc. Three healthy volunteers (one male, 2 females) stated that they had not ingested NSAIDS, ProvexCV®, red wine, red grapes, purple grape juice, or grape jelly for a two week period prior to the blood sampling and were not currently on any prescribed medications. A 19 G-butterfly needle was used for the donation process so that shear forces could be kept to a minimum. 18 mL of whole blood was extracted from each donor and mixed with 2 mL of 3.8% Sodium Citrate solution to achieve a 9:1 ratio. The blood sample was then mixed with equal parts (20 mL) of preservative-free saline. Collagen (Chrono-log) was used as the initiator for aggregation; a 4:1 solution was made using 5% dextrose (Baxter): collagen.

A 1 mL sample of diluted blood was placed into a cuvette (Chrono-log) and then inserted into the sample well of the aggregometer (Chrono-log 590 4-channel impedance whole-blood aggregometer) and allowed to incubate for 5 minutes. 4 µL of the formulation were added to the blood sample during incubation and stirred using a siliconized stir-bar (Chrono-log) placed at the bottom of each cuvette. After the 5-minute incubation period, aggregation was induced using 8 µL of the collagen solution and allowed to occur for 7 minutes. Electrical impedance due to platelet aggregation was measured using the aggregometer and recorded using the AGGRO/LINK Software (Chrono-log).

5. Two different formulations were prepared to compare the effects of different grape seed extracts on platelet aggregation inhibition activity, **Formulation A** and **Formulation B**. **Formulation A** was modified from a formulation set forth in U.S. Patent No. 6,818,233; **Formulation B** is based on a different formulation set forth in U.S. Patent No. 6,818,233. Each formulation differed only in the grape seed extract, and three different grape seed extracts were used. Grape seed extract 1 was purchased from Polyphenolics (Canandaigua, NY) and is extracted from a mixture of ruby red, chardonnay, colombard, and chenin blanc grape seeds. Grape seed extract 2 was purchased from Indena (Milan, Italy), and is extracted from champagne grape seeds. Grape seed extract 3 was purchased from Greenway International (Orem, UT), and is extracted from Muscat grape seeds.

Formulation A contained the following ingredients:

Ingredient	% Formulation	Amount, mg
Grape Seed Extract (1, 2, or 3)	15.8	59
Ginkgo Biloba Extract	13.16	50
Bilberry Extract	13.16	50
Grape Skin Extract	26.32	100
Quercetin	31.60	121
Total	100%	380 mg

Formulation B contained the following ingredients:

Ingredient	% Formulation	Amount, mg
Grape Seed Extract (1, 2, or 3)	20	25
Ginkgo Biloba Extract	8	10
Bilberry Extract	8	10
Grape Skin Extract	24	30
Citrus Extract	40	50
Total	100%	125 mg

6. **Formulations A** and **B** were prepared by dissolving the appropriate amount of each ingredient in 1 mL of solvent. The solvent is a mixture of 300 µL of DMSO (Sigma-Aldrich) and 700 µL of 0.9% preservative-free saline (Abbot

7. The results for **Formulation A** at two different doses are shown below in **Table I.** (NS denotes not statistically significant.)

Table I

Sample Description and dose	**% Platelet Aggregation Inhibition Activity N=3**	**Standard Deviation**	**P value Compared to Formulation A using grape seed extract 1 (at same dose)**	**P Value Compared to Formulation A using grape seed extract 2 (at same dose)**
Formulation A using grape seed extract 1 380mg/L	4.55%	14.32%	p=1 (NS)	p=0.073 (NS)
Formulation A using grape seed extract 2 380mg/L	46.17%	17.98%	p=0.073 (NS)	p=1 (NS)
Formulation A using grape seed extract 3 (Muscat) 380mg/L	83.47%	2.1%	p=0.002	p=0.02
Formulation A using grape seed extract 1 760mg/L	100%	0%	p=1 (NS)	p=1 (NS)
Formulation A using grape seed extract 2 760mg/L	100%	0%	p=1(NS)	p=1 (NS)
Formulation A using grape seed extract 3 (Muscat) 380mg/L 760mg/L	100%	0%	p=1 (NS)	p=1 (NS)

8. The results for **Formulation B** at two different doses are shown below in **Table II:**

Table II

Sample Description and dose	% Platelet Aggregation Inhibition Activity N=3	Standard Deviation	P value Compared to Formulation B using grape seed extract 1 (at same dose)	P Value Compared to Formulation B using grape seed extract 2 (at same dose)
Formulation B using grape seed extract 1 125mg/L	-18.6%	17.6%	p=1 (NS)	p=0.809 (NS)
Formulation B using grape seed extract 2 125mg/L	-15.8%	9.9%	p=0.809 (NS)	p=1 (NS)
Formulation B using grape seed extract 3 (Muscat) 125mg/L	3.5%	5.9%	p=0.106 (NS)	p=0.044
Formulation B using grape seed extract 1 250mg/L	-9.9%	10.0%	p=1 (NS)	p=0.132 (NS)
Formulation B using grape seed extract 2 250mg/L	46.7%	22.3%	p=0.132 (NS)	p=1 (NS)
Formulation B using grape seed extract (Muscat) 250mg/L	77.8%	0%	p=0.001	p=0.035

9. The results from **Table I** and **Table II** demonstrate that Formulations including Muscat grape seed extract exhibit a statistically significant increase in platelet aggregation inhibition as compared to the comparable Formulations using grape seed extracts 1 and 2.

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity and/or enforceability of the instant patent application or any patent issuing thereon.

Dated: 04/07/2006

Erin Stone

STATE OF Idaho)

) ss.

COUNTY OF Bonneville)

Before me this 7th day of April, 2006, personally appeared Erin Stone known to me to be the person whose name is subscribed to the foregoing Declaration, and acknowledged that he executed the same as his free act and deed for the purposes therein contained.

TIFFANY FOSTER NOTARY PUBLIC STATE OF IDAHO

Tiffany Foster
Notary Public